

# *Subcontractor Report*

## **Colloquies on the Yeast Platform Project**

### **Final Summary Report**

Donald L. Johnson  
*Hertford, North Carolina*



# **NREL**

**National Renewable Energy Laboratory**

1617 Cole Boulevard  
Golden, Colorado 80401-3393

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Contract No. DE-AC36-99-GO10337

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NREL Technical Monitor: Min Zhang

Prepared under Subcontract No. LDH-1-31100-01



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# YEAST PLATFORM COLLOQUIA

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## I. Executive Summary

A project for developing a platform yeast was initiated at the National Renewable Energy Laboratory after industry stakeholders and interested parties expressed concern that available organisms for processing sugars from the enzyme sugar platform are inadequate for commercial use. A project vision and strategy were articulated and shared with potential collaborators and other potential stakeholders. Three colloquia were held at various locations across the United States to share ideas and obtain feedback from invitees who represented industry, academia and other potential collaborators. The project was summarized in a pre-colloquy mailing, and fully described at each session.

Voluminous input was obtained. The majority of participants felt that an additional organism was needed because presently available organisms are not suitable for producing commodities such as fuel ethanol, although one industrial attendee opined that present organisms are adequate. Many felt that the proposed funding is inadequate for a project of this magnitude. Some expressed concern over who would manage such a broad-based, diverse project. The need for representative and reproducible substrates was expressed, as was the need for close integration with the enzyme sugar platform project. Other major concerns included that of how intellectual property would be handled, the need of for timely economic sensitivity analysis and whether the objectives would be specific enough.

Major hurdles identified were, (1) understanding pentose utilization, (2) maintaining yeast viability and stability, (3) organizational integration across the various diverse groups and (4) maintaining a strong central focused management.

Recommendations based upon the feedback are that, (1) the OFD should sponsor development of a commercial organism, (2) fuel alcohol from corn stover should be emphasized, (3) a joint development with industrial collaborators should be considered, (4) NREL should continue developing knowledge and tools in collaboration with stakeholders, (5) a consortium with industrial stakeholders should be considered and (6) the project vision and strategy should be appropriately revised.

## II. Introduction

The National Renewable Energy Laboratory wants to develop a platform yeast which would produce platform chemicals (existing and new) from biomass sugars. The need for a yeast stemmed from discussions with industrial stakeholders and interested parties concerning the already initiated enzyme sugar platform project. A vision was developed and articulated, and a plan for achieving the vision drafted. The success of such a project was recognized to depend upon early input and collaboration with industry, academia and other government labs or agencies. The vision and plan were communicated to potential stakeholders and a series of colloquia were planned and implemented. The colloquia were intended to inform the potential stakeholders about the vision, involve them in the planning and strategies and gain their support and potential collaboration in the project. The results obtained from the colloquia are presented in this report.

## III. Objectives.

The objectives of the colloquia were to:

- a. Describe the Yeast Platform project,
- b. Obtain input and hear concerns from potential stakeholders,
- c. Thoroughly define relevant issues,
- d. Examine project hurdles, and
- e. Strengthen the vision and strategy based upon the input.

## IV. Colloquia

### a. Structure

A colloquy was held in each of three cities and scheduled to make it easy for participants arrive and depart one 24 hour period. Sites were located geographically to maximize attendance. Thus, the Denver International Airport served the West, Chicago's O'Hare International Airport served the Midwestern invitees and Dulles International Airport (Washington, DC) served the Eastern seaboard and provided access to those who couldn't attend an earlier session. Starting time was 9:30 a.m. at each, lunch was served at or near the site and a 3:00 p.m. adjournment was guaranteed. The Denver, Chicago and Washington meetings were held July 17, July 20 and July 25, respectively.

Invitations were tendered to industrial folks who were either active in fermentation or interested in chemicals and fuels from biomass, to academic leaders in yeast research and to laboratories working in the yeast area. The goal was to have 3-4 times as many industrial attendees as academic, and to have 15 to 24 attendees per session. These people were invited by telephone,

followed up with an Email with the purpose of the colloquy and a questionnaire that they were to answer and bring to the meeting.

As normally occurs, not all that were invited accepted, and not all who accepted the invitation actually attended a colloquy.

Attendees at each colloquy are listed in Appendix A.

b. Agenda.

The agenda for each colloquy was identical, although the discussions developed a different character at each location. After introductions, the meeting goals, agenda and ground rules were presented. Following Fuel Development and Biofuels Technical Program overviews by DOE Headquarters staff and NREL Technical Managers, the Yeast Platform Vision was presented by the project leader, Min Zhang. This latter was a formal presentation describing the project for the attendee's information. A copy of the power point program notes is attached as Appendix B. A discussion on the vision and concerns with it followed. After lunch discussion continued where improvements were solicited and additional concerns examined. The meetings ended with a summary by the discussion leader and a presentation of plans for follow-up and next steps. The agenda which was sent to each invitee, is attached as Appendix C.

Ground rules were established to keep the discussions focused on the Yeast Platform project, and emphasized at the beginning of each session. They were:

- This was to be an informal, freewheeling discussion,
- No entity dominates the discussion-we want to hear from everyone,
- Grind no axes-focus on project, and
- Three (3) minute limit/person-entity/point.

The word "entity" was used because some organizations had more than one representative.

There were also four starting assumptions which were not open for debate:

- There will be fuel ethanol from biomass,
- "Enzyme Sugars" from hydrolyzed biomass will be available,
- The biomass will initially be corn stover (switch grass, trees, etc. later), and,
- The enzyme sugar stream will contain the five sugars, glucose, mannose, galactose, xylose and arabinose.

The “next steps” included the discussion leader summarizing the results of the three colloquies and Emailing to each participant for their review and additional input. They were also asked for their recommendation on how to proceed with the project.

V. Feedback on Current Project Vision.

Responses to the questionnaire, discussed at the colloquy and received afterwards, were generally favorable to the Yeast Platform Project. Several, especially those involved in developing and scaling up biochemicals, did not feel that the resources allocated are adequate for the magnitude of the undertaking. However, two manufacturing firms and at least one enzyme development company felt that the project could be done with the proposed allocation and time frame, *if they were given the whole project and resources*. There was also concern that the timeline and need for good integration of research by experts across diverse technical lines would be better managed by industry. The expected concerns about handling intellectual property and competitive advantage were expressed by several. Only one respondent recommended how to proceed. That was for NREL to coordinate the basic research, and have the Renewable Fuels Association provide technical monitors to oversee project direction and development.

a. On the question “is the project a good idea,” nearly all participants agreed that the Yeast Platform Project was a good idea and that a yeast was needed for industrial fermentations. There was one dissenter who expressed that there were already organisms (bacteria) which work and should be used. However, industrial attendees who performed commercial fermentation operations preferred yeast. A sampling of comments in answer to the question “Do you think that the proposed project is generally a good idea?” is included in Table 1, below.

Only one participant of the three sessions who was negative to the Yeast Platform. One other industrial participant, commenting in a follow-up correspondence, did not think a consensus was achieved that “industry needs a yeast.” He perceived the outcome to be that if the existing corn wet milling industry is DOE’s target then the statement is true. If a company is building a new biomass-to-ethanol facility, then non-yeast organisms may, with some level of improvement, work fine.

While the corn refining industry would benefit from an efficient organism, just as other industries have benefited from spin-off from NASA and DOD projects, for example, fiber is not a primary goal. If all the fiber were converted to biomass sugars, it would represent less than 10% of the potential of one-half the corn stover available! A corn stover to ethanol operation could occur first outside the corn refining industry, given avail-ability of an appropriate process.

b. Major hurdles expressed included substrate toxicity, yeast physiology and organizational. In addition to the substrate toxicity, which is well known in the biomass arena, efficient utilization of pentoses and understanding pentose utilization were the most frequently expressed technical issues.



Maintaining yeast viability, stability and robustness while engineering the requirements into it would be a challenge. A genetically engineered organism would be required to achieve the vision, according to the participants, which would be an issue if a food product were involved (such as using fermentation residue in animal feed). That industrial participants emphasized organizational

Table 1. Selected Responses to the Question of Whether Another Organism is Needed for Fermenting Biomass Sugars.

Entity	Comment
Ag Processor	"It seems clear that a yeast, specifically an engineered <i>Saccharomyces</i> , is required to achieve the goal of efficiently producing fuel ethanol and bio-based products from biomass sugars."
Ag Processor	"We need another (organism), a very robust one."
Ag Processor	"In my opinion, creating a yeast platform/focus at NREL is a much needed endeavor."
Ethanol Producer	"Unless we have an efficient fermentation organism, the pretreatment work and enzyme sugar platform are meaningless."
Biotech Firm	"Yes-need GRAS platform organism and should be improvable. May be applicable to a broader range of products (at least as a starting point)."
University	"Yes I do.... <i>Saccharomyces</i> is arguably the microorganism best suited scientifically to this vision because it is so well studied and there is an aggressive, highly interactive community of outstanding scientists available to do the work. Finally, a state of the art suite of genetic tools unmatched in any organism is available."
Industrial Company	"The project as proposed is not a good idea, and may conflict with legislation and established policy, especially involving antitrust and small business. DOE and USDA have already funded successful research resulting in milestone patent US#5000000 which teaches an organism developed to ferment all the sugars derived from biomass."
Government Lab	"Yeast essentially don't handle arabinose. <i>E. coli</i> are subjected to phage infections, a problem with all microorganisms at this point."

hurdles, whereas academics focused on technical hurdles was not surprising. Both groups, however, agreed that a strong, central management would be needed to keep the project focused among the diverse groups involved.

- c. The major concern expressed by both academicians and industry was whether the program was adequately funded for the task presented. Two industrial representatives felt that proposed funding is a factor of ten low. Other industrialists, felt that the project is doable within the resources proposed, if it were industry led and leveraged internally. Another recurring theme among the concerns, predominately from industry, was who has access/ownership of the intellectual property resulting from the work. How would all the pieces of the research be integrated in a timely and

open manner was also a concern. That the goals should be more specific was also a concern, primarily from academia.

- d. Although the majority of both academic and industrial participants felt that the time frame was realistic, it was a slim majority. Those dissenting felt that either the resources were not adequate or the goals had to be more specific. Again, some major industrialists felt that the objectives could be met within the time and resources mentioned, with appropriate sub-contracts and increases of in-house resources, *provided industry were contracted to do it.*
- e. Most of the responses, academic and industry, were for NREL's role to be that of coordination. Outside research should be subject to the same reporting and review requirements as in-house research at NREL. It is important to maintain transparency, said one industrial respondent, so that flawed research can be quickly uncovered and dealt with. At least one industrial firm commented that NREL should stay out of the business of organism development unless industry chooses to invest in a project to develop a yeast.
- f. In general, industry would support a consortium "under appropriate circumstances." A range of \$50,000 to \$100,000 per member was proposed and was not objectionable. They would join if the value returned would be greater than the investment. However, few believed that the consortia route was the best way to achieve the goals. Some would support the effort and would coordinate with the Corn Refiners Association and National Corn Growers Association to insure funding (funding level was not stated). The academicians would support the project with research and intellectual input if funded, which was not surprising.
- g. For the most part, industry would consider entering a CRADA with NREL once initial success was demonstrated *if it served their interest*. For some it would be in demonstrating the organism under realistic conditions, or fermenting corn refiners fiber. One would consider entering a CRADA with NREL in the Biofuels and/or Chemicals area once feasibility or reasonable progress is demonstrated. Of course, most academicians would consider entering a CRADA *with and industrial partner*. Some academicians did not feel that the CRADA question applied to them.
- h. No respondent offered a specific product. Organic acids, and general chemicals or intermediates were offered up. One industrial company thought NREL should focus on developing enabling tools for organism development. Two fermentation alcohol producers said that fuel alcohol should be the priority.
- i. There was no consensus on a change in project vision. Among the industrial participants, opinions ranged from a longer timeline, should have started in combination with the enzyme platform project, focus on development of enabling tools helpful to industrial researchers to broaden the metabolic capability of yeast to include other sugars, fatty acids, alkanes and glycerol. Academicians suggested leaving an option for two or three organisms (preferably yeasts), make the goals more

specific and realistic and more basic research, perhaps in the area of “bio-prospecting.”

- j. Nearly everyone volunteered interest in serving as a member of an advisory panel.

Additional comments included:

- “You need to bring in a diverse group of partners who are committed to success of the program and not view it as another source of funding.”
- A priority should be the influence of economics on each of the goals and determine what makes the most sense-may need to integrate all the goals because of the cross-cutting nature. The nature of DOE which is forced to fund and divide into efforts, this mechanism almost frustrate the effort.
- “It would be difficult for me to get our company to buy in on a commodity product (e.g. EtOH), but possible for fine/specialty chemicals, in the context of platform organism development.
- “It is likely that unless awarded a major program role such as managing the program most companies will ‘track’ this program but probably do little more initially.”
- “I would like to see the RFP worded so that the goals are very specifically laid out. I think having a centralized resource at NREL that provides the following core services to all participants would be an excellent way to go. This resource center would include: Hydrolysate repository, yeast strain repository, centralized bioassay service and centralized database accessible via intranet to consortium members.”
- “Industry has a very good understanding (or at least they soon will when they start putting the effort into such a project) of the detailed economic sensitivities and tradeoffs involved in such a complex process-details that are not available to those working outside of industry due to the proprietary nature of such models.”
- Paraphrasing one industrial company’s interest, ‘interested in fundamental/technology elements-depends upon structure of effort, academic side is intriguing, it looks like a good platform.’

## VI. Conclusions

- a. The yeast platform project was summarized in the pre-colloquy mailing, and described fully at each colloquy.
- b. Voluminous input was obtained at each colloquy, although each site developed its own character due to the mix of the attendees. Denver participants were mostly academics or research types, while Chicago and Washington had a preponderance of industry representatives. The majority of participants, academic and industrial, felt that the another organism is needed in industry and that the Yeast Platform Project is a good idea. There was one dissenter, however, who that felt enough organisms are available to convert enzyme sugars. The industry viewpoint is that the available organisms are not suitable for producing a high

volume, low margin commodity such as ethanol. Concerns were heard and recorded, as well as invited in follow up correspondence.

- c. Relevant issues expressed include:
  - i. Projected funding is inadequate for the magnitude of the task.
  - ii. Who should manage the program
  - iii. A reproducible synthetic substrate, as well as “real” substrates will be needed, and close integration with the enzyme sugar platform required.
  - iv. Realistic economic sensitivity analysis will be needed on a timely basis.
  - v. Academics felt that the project needed more specific objectives.
  - vi. How will the intellectual property be handled.
- d. Major hurdles, in addition to the substrate toxicity, which is well known in the biomass arena, were expressed as:
  - i. Efficient utilization of pentoses and understanding pentose utilization,
  - ii. Maintaining yeast viability, stability and robustness while engineering the requirements into it,
  - iii. A genetically engineered organism would be required to achieve the vision, which would present a hurdle if a food product were involved (such as using fermentation residue in animal feed).
  - iv. Industrial participants emphasized organizational hurdles, whereas academics focused on technical hurdles.
  - v. Both groups, however, agreed that a strong, central management would be needed to keep the project focused among the diverse groups involved.
- e. There was no consensus on a change in project vision. Some thought the vision is fine as is, others suggested extending the timeline, focusing on development of enabling tools, broadening the metabolic capability of yeast, leaving an option for two or three organisms, making the goals more specific, and more basic research.

## VII. Recommendations

### **a. The Office of Fuels Development (OFD) should sponsor development of a commercial organism.**

The people who are presently conducting commercial fermentations all agreed that a yeast is needed because the existing organisms are not adequate for industrial, commodity-type fermentations such as would be needed for fuel alcohol production.

### **b. The emphasis of the program should be the production of fuel alcohol from the sugars derived from corn stover.**

This should be emphasized and differentiated from fermenting the fiber residual in a corn refining plant. Spin-off may be helpful to the corn refiners, but the two raw materials are distinct from one another. Moreover, fermenting all the corn fiber would represent less than a tenth of the alcohol available from fermenting half the corn stover produced. This message should be more widely publicized.

- c. Consider a joint development, partnering with a yeast developer and a yeast user.** Structure an RFP so that strong proposals can come from, for example, Maxagen/ GPC, Diversa/Tate&Lyle or Alltech/Cargill (The foregoing are presented only as examples). Two companies stated that they could do the project within the time frame and resources indicated, if it were internalized into their respective organizations where they could leverage the dollars received.
- d. If OFD decide that they should not develop commercial organisms, then a consortium should be formed.** Nearly all participants are interested in the project, but none would commit to funding/collaboration (except academicians for collaborations) at an early stage of the project. If priced right, many companies would likely join to keep up with the program. A steering committee of consortium members would be helpful to keep realism in the development.
- e. The consortium project should focus on developing tools and information, tasks 1,2 and 3 of the original plan.**
- f. The NREL project vision should be appropriately revised to reflect the change in scope.**

VIII. Appendices

- a. Attendees
- b. Yeast Platform Project power point presentation.
- c. Colloquy Agenda
- d. Sample questionnaire

## Appendix A

### Yeast Platform Colloquia ATTENDEES

#### Denver Colloquy

Amit Vasavada	Diversa
Don Timbur	Genencor
Jim Mattoon	U Colorado – Colorado Springs
Matt Tobin	Maxygen
Frank Rosenzweig	U of Florida
Keith Villa	Coors
Sharon Shoemaker	U of California – Davis
David Ogtydziak	U of California – Davis
David Nunn	Diversa
Paul Levine	Enogen
Mark Finkelstein	NREL
Bob Wooley	NREL
Cindy Riley	NREL
Gerson Santos-Leon	DOE
Valerie Reed	DOE
Amy Miranda	DOE

#### Chicago Colloquy

Eric Dennison	ADM
Doug Cameron	Cargill
Pearse Lyons	Alltech
Tim Arthur	Alltech
Karl Dawson	Alltech
Ronan Power	Alltech
Chris Ryan	Cargill-Dow
Sergi Johal	GPC
Streve Lewis	Broin
Marion Bradford	Tate & Lyle
Rod Bothast	USDA
John Nghiem	ORNL
Vassily Hatzinmanikatis	Northwestern Univ.
Mike Ladisch	Purdue Univ.
Nancy Ho	Purdue Univ.
Sabrie Ozan	Washington U
Mark Johnson	NREL
Stan Bower	NREL
Bob Wooley	NREL
Arjun Singh	NREL
Valerie Reed	DOE

## Appendix A

### Washington Colloquy

Bob Dorsch	Dupont
Steve Picataggio	Dupont
Brian Foody	Iogen
Brent Erickson	BIO
Joe Glas	BCI
Tim Presnell	West Vaco
Jennifer Snyder	CRA
Brian Davison	ORNL
George Laurance	Fleischmann Yeast
Fred Sherman	U of Rochester
Dan Fraenkel	Harvard Medical School
Tom Jeffries	U of Wisconsin
Jeff Boeke	Johns Hopkins
Mark Finkelstein	NREL
Arjun Singh	NREL
Stan Bower	NREL
Valerie Reed	DOE
John Ferrell	DOE
Richard Moorer	DOE
Amy Miranda	DOE

## Appendix B



### **Yeast Platform Project**

*Presented by  
Min Zhang*

National Bioenergy Center  
Biotechnology for Fuels and Chemicals Division  
National Renewable Energy Laboratory

July 25, 2001  
Washington DC Colloquy

### **Yeast Platform Project Description**

- **To develop vision for the yeast platform project and identify key partners**
- **To develop yeast as a platform organism for the production of bioenergy and biobased products from biomass sugar streams**
- **To develop advanced genetic engineering tools and knowledge to enable us to address a multitude of industrial concerns in biocatalyst development**



## **A Vision for Development of the Yeast Platform**

*By 2007 a commercially viable yeast platform will be available for converting all five biomass sugars in high yield to selected platform fuels and chemicals. Tools and knowledge will be available to genetically manipulate the organism for producing a number of bio-based products including ethanol, organic acids, diols and other alcohols as selected by industrial collaborators.*

## **Background**

### **Why Yeast?**

- Proven robustness in industrial fermentation processes
- Well accepted in industry
- Strong feedback from industry customers
- GRAS (*Saccharomyces*), co-product value as feed
- Thermotolerant (~50 °C)
- Acid tolerant (~pH 3.5)

## What Can Yeast Currently Do in Terms of Fermenting Biomass Sugars?

### Ethanol

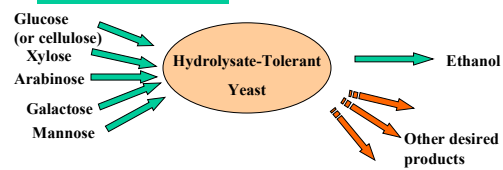
- Ferment glucose to ethanol at a high rate and high yield
- Ferment mannose to ethanol at a fairly high rate and high yield
- Ferment galactose to ethanol at a slow rate and high yield
- Ferment xylose to ethanol but at a low rate and relatively low yield, not robust in fermentation of hydrolysate
- Do not ferment L-arabinose to ethanol

### Others

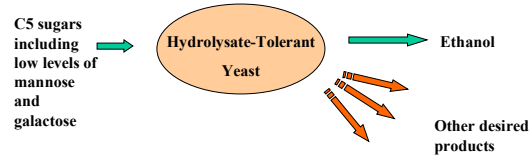
- Produce lactic acid from glucose
- Produce xylitol from xylose
- Other products

## Overview of Platform Yeast and Platform Chemicals

### A. All biomass sugars



### B. Biomass sugars from hemicellulose



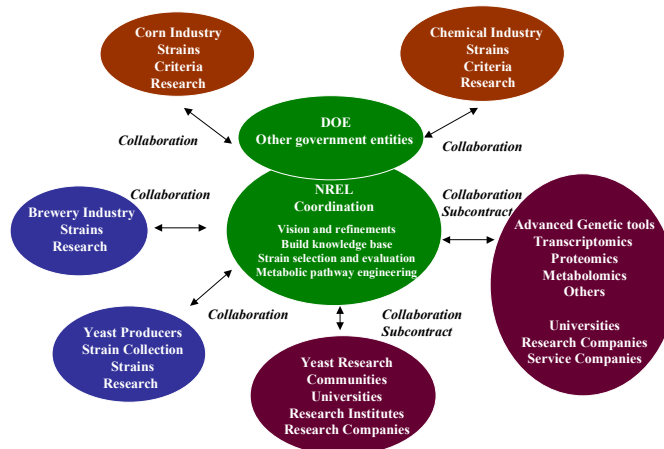
### **Technical Challenges**

- **Enhance hydrolysate tolerance**
- **Utilization of all sugars at high rates**
- **Production of selected chemicals at high yield and titer**

## Advanced Tools

- **Transcriptomics**
- **Proteomics**
- **Metabolomics**
- **Flux Analysis/Metabolic Modeling**
- **Directed Evolution**

## Key Relationships for the Yeast Platform Project



### The Plan to Reach-out for Collaborations (Calendar Year 2001)

	May	June	July	Aug	Sept.	Oct.	Nov.	Dec
Preparation for the colloquies	_____							
Colloquy 1			—					
Colloquy 2			—					
Colloquy 3			—					
Report				—				
Follow-up meeting				—				
RFP out				—				
Review proposals					—			
Subcontract placement						—	—	—

### The Specific Research Subjects (Hurdles)

- Understand and Improve Hydrolysate Tolerance
- Understand and Improve Uptake of C5 Sugars
- Enhance C5 Utilization Rate
- Convert All Biomass Sugars to Desired Product
- High Yield
- Process Robustness
- Strain Evaluation (Physiology)
- Genetic Tools
- Production of Cellulase Enzymes in Host Strains

### **Understand and Improve Hydrolysate Tolerance**

- Understand toxicity of the hydrolysate-chemically, biochemically and through application of functional genomics/proteomics
- Select most tolerant strains to the hydrolysate
- Adaptation
- Devise strategy to reduce the toxicity
  - Pretreatment
  - Introduce detoxification pathways

### **Understand and Improve Uptake of C5 Sugars**

- Kinetic measurement
- Cloning and characterization of pentose transporters
- Expression of better transporters
- Engineering better transporter via protein engineering or directed evolution
- Classical mutagenesis and selection using chemicals

### **Enhance C5 Utilization Rate**

- **Overexpression of necessary genes as guided by flux analysis, transcriptomics, proteomics and/or metabolomics**
- **Eliminate other unnecessary pathways**

### **Convert All Biomass Sugars to Desired Product**

- **Utilize all five individual biomass sugars**
- **Utilize a mixture of biomass sugars and capable of fully converting the utilized sugars to a desired product or products**
- **Convert all sugars at faster rates**
- **Deregulate the glucose catabolite repression (xylose, galactose and arabinose (?))**
- **Engineer a new pathway as needed for the desired product**
- **Flux analysis, transcriptomics, proteomics and/or metabolomics, and directed evolution are tools to be applied in this area for further metabolic engineering**

## **High Yield**

- **Pathway optimization**
- **By-product elimination**
- **Understanding oxygen regulation**
- **Elimination of oxygen regulation if needed**
- **Flux analysis, transcriptomics, proteomics and/or metabolomics and directed evolution are tools to be applied for further metabolic engineering**

## **Process Robustness**

- **Choose a robust host organism**
- **Re-evaluated the organism following pathway engineering to demonstrate that robustness has not been compromised**
- **Complete utilization of all five biomass sugars is also a key to ensure process robustness because it leaves no residual sugar to invite other microorganisms into the fermenter**



### **Strain Evaluation (Physiology)**

- **Strain evaluation will be needed throughout this project to help determine progress and where the problems lie**
- **Flux analysis, transcriptomics, proteomics and/or metabolomics can guide to further strain improvement**

### **Genetic Tools**

- **Genetic markers**
- **Vectors**
- **Promoters**
- **Transformation methods**
- **Gene insertion methods**

## Production of Cellulase Enzymes in Host Strains

- Express cellulase genes in yeast
  - Extracellular
  - Cell surface

### Estimated Resources for the Yeast Platform Project

	FY02	FY03	FY04	FY05	FY06	FY07
Continue Stage A screening and selection activities	0.5	0.5	0.5			
Understand and improve hydrolysate tolerance	2	2	2	2	1	1
Understand and improve C5 uptake	3	3	3	3	1	1
Enhance C5 utilization	4	4	4	4	3	3
Convert all biomass sugars to desired product	1	2	2	2	3	3
High yield	1	2	2	3	4	4
Process robustness	0.5	0.5	0.5	0.5	0.5	0.5
Strain evaluation (physiology)	2	2	2	2	2	2
Genetic tools	3	2	1	1	1	1
Advanced tools	7	6	7	6.5	8.5	8.5
Transcriptomics						
Proteomics						
Metabolomics						
Directed evolution						
Flux analysis						
Genome sequence if needed (\$ million)		\$2				
Project Management	1	1	1	1	1	1
<b>Total FTEs</b>	<b>25</b>	<b>25</b>	<b>25</b>	<b>25</b>	<b>25</b>	<b>25</b>
<b>Estimated Budget (\$ million) (\$200k/FTE)</b>	<b>\$5</b>	<b>\$7</b>	<b>\$5</b>	<b>\$5</b>	<b>\$5</b>	<b>\$5</b>

## Appendix C

### Yeast Platform Project Colloquy

#### Agenda

9:30 a.m. – 3:00 p.m.

9:30-9:35	Introductions	D. Johnson
9:35-9:45	Purpose of Meeting <ul style="list-style-type: none"><li>• Meeting Goals</li><li>• Review Agenda and Ground Rules</li></ul>	D. Johnson
9:45-10:30	Background <ul style="list-style-type: none"><li>• DOE Program Perspective</li><li>• Biofuels Technical Overview</li><li>• Yeast Platform Vision</li></ul>	DOE Hq Staff (10 min) R. Wooley (10 min) M. Zhang (25 min)
10:40-12:00	Discussion Original Project Vision and Concerns <ul style="list-style-type: none"><li>• Is the project needed? By who? What do they need? Goal: Confirm general project need, direction, and emphasis.</li><li>• What are concerns, issues, hurdles, and showstoppers with the project vision?<ul style="list-style-type: none"><li>- Scientific/Technical</li><li>- Organizational/Institutional</li></ul></li></ul> Goal: Develop comprehensive list of issues etc. identified by participants. This may be best accomplished using a brainstorming technique.	All
12:00-12:30	Lunch Served (will work through lunch (if needed)).	
12:30-2:30	Discussion: Improvements and Revised Project vision <ul style="list-style-type: none"><li>• Suggestions for improving the Project<ul style="list-style-type: none"><li>- Scientific/Technical Strategy</li><li>- Organizational Approach</li></ul></li></ul> Goal: Revise and strengthen the project strategy and organization.	All
2:30-2:45	Meeting Summary	D. Johnson
2:45-3:00	Plans for Follow-up and Next Steps	D. Johnson

## Appendix D

### Stakeholder Questionnaire DOE/NREL Yeast Platform Project

#### Feedback on Current Project Vision

1. Do you think that the proposed project is generally a good idea? Why or Why not?
2. What do you see as the major hurdle(s) to realizing the vision? (Scientific/Technical and / or Organizational)
3. What concerns do you have with the project? Please list.
4. Is the timeframe realistic? Why or why no?
5. What do you see as the proper role for NREL (a DOE National Laboratory) in this project?

#### Your Organization's Perspective:

6. What would be your organizations level of support for the project?
7. Would your organization have interest in joining a consortium, with annual dues to partially support the effort, in which members receive frequent updates and participate in the direction of the research agenda?
8. Would your organization consider entering into a Cooperative Research and Development Agreement (CRADA) with NREL when initial success is shown? If so, in what general area?
9. What particular product or products (in priority order) would be of most interest to your organization?
10. If your organization could make one change to the project vision, what would it be?

Appendix D  
Continued

Future Involvement:

11. Would you be interested in serving as a member of an advisory panel to review progress and help guide the research effort?

Additional Thoughts, Comments or Suggestions:

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